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The effect of different trophic modes on lipid accumulation of *Scenedesmus quadricauda*

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ABSTRACT

In this study, the effects of different carbon sources on cell growth and lipid accumulation of *Scenedesmus quadricauda* were investigated. Results showed that *S. quadricauda* could grow on photoautotrophic, heterotrophic and mixotrophic modes. The lipid yield of *S. quadricauda* was much lower in the culture containing $NaHCO_3$ as only carbon source, while CO_2 and glucose concentration significantly influenced cell yield and lipid accumulation in photoautotrophic and heterotrophic culture, respectively. Furthermore, lipid content of *S. quadricauda* in mixotrophic culture (33.1% of cell dry weight) was much higher than that in photoautotrophic and heterotrophic cultivation (14–28%). Therefore, upon comparing these three trophic modes, present results revealed mixotrophy was the optimal culture method for *S. quadricauda* to produce lipid. Besides, it was a feasible and promising strategy to culture *S. quadricauda* using starch wastewater as raw material, which could reduce chemical oxygen demand (COD) of wastewater and the cost of biodiesel production.

1. Introduction

In last several decades, due to over consumption of fossil fuels such as petroleum fuels, coal and natural gas, they are to be exhausted in the future and the resulting energy crisis will become one of the largest challenge of the 21st century (Vasudevan and Briggs, 2008). Meanwhile, global warming is considered to be associated with burning fossil fuels. Biodiesel, an environmental beneficial and renewable resource, is well known as an alternative diesel fuel (Miao and Wu, 2006). Due to lack of conventional raw materials for biodiesel production, such as plants oils and animal fats, renewable microbial oils has been widely regarded as a potential material. Using microorganisms to produce lipid has many merits, such as high lipid content, short production period and less requirement of labor force (Brennan and Owende, 2010).

Recently, the study of biodiesel production from microalgae has become a hot topic with carbon dioxide emissions becoming an increasing concern as an environmental issue (Gouveia and Oliveira, 2009; Pruvost et al., 2009). Microalgae are considered as an attractive source for biodiesel production due to their high lipid content, photosynthesis efficiency and CO_2 reduction efficiency (Xiong et al., 2010). However, there are still some challenges in photoautotrophic culture of microalgae for biodiesel production. For example, in photosynthesis system, microalgal cells grow slowly and contain low lipid content. Heterotrophic microalgae can obtain higher lipid content and biomass productivity, but the extra feedstock cost is also higher and the energy transformation efficiency is low (Li et al., 2010). Some microalgal species can conduct mixotrophy in the culture containing both inorganic and organic substrates (Ip and Chen, 2005; Sun et al., 2008) which are simultaneously assimilated, with both respiratory process and photosynthesis occurring concurrently (Kaplan et al., 1986; Lee, 2004). According to a study (Marquez et al., 1993), the growth rate of mixotrophic culture is the sum of the photoautotrophic growth and heterotrophic growth. The mixotrophic growth of some microalgae produced 3–10 times more biomass yields as compared with phototrophy (Bhatnagar et al., 2011). Besides, it is reported that the *Chlorella protothecoides* in mixotrophic culture can accumulate 69% higher lipid productivity with 61.5% less release of CO_2 relative to typical heterotrophic growth (Xiong et al., 2010). Meanwhile, there are few references related to lipid production using *Scenedesmus quadricauda* strains and fewer reports covering trophic modes of *S. quadricauda*. Thus, one of the aims of this research is to confirm trophic types of *S. quadricauda*. In this study, *S. quadricauda* was cultivated in different carbon sources, and the effects of trophic modes on cell growth and lipid accumulation of *S. quadricauda* were investigated as well. Additionally, the best culture mode of *S. quadricauda* for lipid production was also determined in the research.

Moreover, according to report (Lu et al., 2009), amount of wastewater produced from starch manufacturing has been increasing annually and has already reached over 20 million tons in china. It must be discharged after some treatment processes because of

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its high COD content (around 40,000 mg L^{-1}). Meanwhile, starch wastewater also contains some useful compositions, such as organic nutrients, which can be consumed by microorganism (Huang et al., 2005; Xue et al., 2010). On the other hand, the microalgae *Scenedesmus* species was cultivated in artificial wastewater (Voltolina et al., 1998) or fermented swine wastewater (Kim et al., 2007) to remove nitrogen, phosphate and other contaminants. In a study, *Scenedesmus* sp. could also grow in secondary effluent wastewater to accumulate lipid (Li et al., 2010). It is a promising approach for handling energy crisis and industrial pollution to combine lipid production and wastewater treatment. Therefore, the study of *S. quadricauda* culture for lipid synthesis using starch wastewater as feedstock was carried out in this paper.

2. Methods

2.1. Strains and culture medium

The *S. quadricauda* strain was stored in Beijing Key Lab. of Bioprocess at Beijing University of Chemical Technology after being provided by Institute of Hydrobiology, Chinese Academy of Sciences.

Algae medium BG-11 composition was: Citric acid 6.0 mg L^{-1}, Ferric ammonium citrate 6.0 mg L^{-1}, EDTA 1.0 mg L^{-1}, $NaNO_3$ 1.5 g L^{-1}, $K_2HPO_4{\cdot}2H_2O$ 0.051 g L^{-1}, $MgSO_4{\cdot}7H_2O$ 0.075 g L^{-1}, $CaCl_2$ 0.024 g L^{-1}, Na_2CO_3 0.02 g L^{-1}, A5 trace mineral solution 1.0 mL L^{-1}. The composition of A5 was: H_3BO_4 2.86 g L^{-1}, $MnCl_2{\cdot}4H_2O$ 1.81 g L^{-1}, $ZnSO_4{\cdot}7H_2O$ 0.222 g L^{-1}, $Na_2MoO_4{\cdot}2H_2O$ 0.391 g L^{-1}, $CuSO_4{\cdot}5H_2O$ 0.079 g L^{-1}, $Co(NO_3)_2{\cdot}6H_2O$ 0.049 g L^{-1}. The initial pH was adjusted to 6.5–7.5.

2.2. Culture conditions for the three trophic modes

2.2.1. Photoautotrophic culture

S. quadricauda was inoculated from plate culture to 500 mL Erlenmeyer flasks containing 150 mL of algae medium. The flasks were placed in the constant temperature shaker and exposed to light at around 73 μmol m^{-2} s^{-1}. The incubation temperature was 27 °C. The algal cells were transferred into 2.3 L air lift photobioreactor (30 cm length, 10 cm diameter) at the logarithmic phase with 10% (v/v) inoculum. The alga was cultivated in the medium with air aeration and different concentrations of $NaHCO_3$, or with gas mixture comprising air and different concentrations of CO_2 under 27 °C and 73 μmol m^{-2} s^{-1}. The flow rate of aeration was set as 1.2 L min^{-1} with air or CO_2 concentration of 2% and 5%, respectively. Aseptic air filtered (0.22 μm) was accessed to the bottom of bioreactor through a pipeline and was dispersed by an air distributor. Light intensity was measured from the light-attached surface of the photobioreactor using a light-meter. Different concentrations of CO_2 aeration were mixed with air and pure CO_2, and adjusted by gas flow meter.

2.2.2. Heterotrophic culture

The heterotrophic algal cells were obtained after *S. quadricauda* was cultured in medium containing glucose for a certain period. The heterotrophic culture was incubated at 27 °C in Erlenmeyer flasks at a rate of 140 rpm. The glucose concentrations of the medium were 0, 3, 5 and 8 g L^{-1}, respectively.

2.2.3. Mixotrophic culture

The green cells of *S. quadricauda* were obtained in heterotrophic culture as described above. At the log-phase growth, the algal cells were inoculated in 2.3 L photobioreactor with algal medium containing different concentration of glucose and air mixed with CO_2 aeration. The glucose concentrations of the medium were 0, 3 and 5 g L^{-1}, respectively. The culture conditions were set as 1.2 L min^{-1} of mixed gas containing 2% CO_2 at 27 °C, and light intensity was approximated to 73 μmol m^{-2} s^{-1} at the surface of photobioreactor.

2.3. Determination of biomass and glucose concentration

Biomass concentration of *S. quadricauda* was determined by optical density measurements at 680 nm using a UV/visible spectrophotometer (UV-2000, Unico, USA). Each sample was diluted to keep an absorbance within the range 0.1–1.0 if optical density was greater than 1.0. The cells obtained from 40 mL of broth after centrifugation at 3000*g* for 10 min were washed twice with distilled water and dried to constant weight at 60 °C. The dry cell weight (DCW) corresponded to OD_{680} value by a regression equation: $y = 0.4901x - 0.0478$ ($R^2 = 0.9951$, $P < 0.05$), where y (g L^{-1}) is the DCW, x is the absorbance of broth at 680 nm. Glucose concentration was evaluated using a glucose biosensor (SBA-40C, Biological Institute of Shandong Academy of Sciences). The principle of this detection method was that glucose was catalyzed by glucose oxidase immobilized on membrane and released H_2O_2. Then the metal electrode was touched by the product of H_2O_2 to create certain electric current signal which had a linear relationship with glucose concentration. A certain volume of broth was centrifuged (3000*g* for 10 min) and then supernatant was diluted to certain concentration sample with distilled water. Finally, 25 μL of sample was needed to detect glucose concentration.

2.4. Determination of lipid content

Lipid content was determined by modified method according to the procedure of Bligh and Dyer (1959). Cells were harvested by centrifugation, washed twice with distilled water, and then lyophilized using freeze drier. The dry samples were pulverized in a mortar and extracted using mixture of chloroform: methanol (2:1, v/v). About 3 mL of solvents were used for 0.1 g of dried samples in each extraction step. After agitating the mixture for 1 h at room temperature, the samples were centrifuged at 3000*g* for 10 min. The liquid phase was transferred into test tubes which were weighed beforehand. The process was repeated three times so that the entire lipid was extracted. The combined solvent phase was evaporated at 70 °C and the test tubes were dried completely before weighed again.

2.5. Analysis of lipid composition

Lipid components were analyzed by a gas chromatograph (GC-2010, SHIMADZU, Japan). And the analysis conditions were listed as following: flame ionization detector (FID) 350 °C; column DB-1ht (J&W Scientific, USA), 30 mm (length) × 0.25 mm (inner diameter) × 0.1 μm (thickness); PTV sample entrance (33 cm/s); diffluent ratio 1:5; carrier gas: N_2.

2.6. Determination of CO_2 concentration

The CO_2 concentrations in original gas mixture inlet and exhaust gas outlet were measured accurately using a gas chromatography (GC-2014C, SHIMADZU, Japan) equipped with a thermal conductivity detector (TCD) and a molecular sieve (TDX-01) packed 2 m × 3 mm stainless-steel column. The operational conditions were as follows: injector, detector and oven temperature were 120, 160 and 140 °C, respectively. The carrier gas, argon, was administered at a flow rate of 40 mL min^{-1}.